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The Pharmacology of 1-Phenyl-2-Propylaminopentane (PPAP), a Deprenyl-Derived New Spectrum Psychostimulant

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Abstract—The peculiar tyramine uptake inhibitory effect of (–)deprenyl prompted structure-activity relationship studies aiming to develop new spectrum central nervous system stimulants which are devoid of MAO inhibitory potency and operate *de facto* as indirectly acting, nonreleasing sympathomimetics. Of the derivatives synthesized for this purpose, 1-phenyl-2-propylaminopentane (PPAP) was selected as the reference substance and its pharmacological spectrum is presented. PPAP is taken up by the catecholamine axon terminal membrane and the vesicular membrane but it is devoid of catecholamine-releasing property. As a result, PPAP is, by interference, a potent inhibitor of the uptake of indirectly acting sympathomimetic releasers and of the catecholamine transmitters. This was proved, on the one hand, by measuring the uptake of [^{14}C]PPAP into the catecholaminergic axon terminals and the inhibition of the uptake of [^{3}H]noradrenaline and [^{3}H]dopamine by PPAP in the rat brain, and, on the other hand, on the pulmonary artery strip of the rabbit and, *in vivo*, using the rat nictitating membrane as a detector.

PPAP increases motility at 2 mg/kg and, in contrast to amphetamine, inhibits it at very high doses (50 mg/kg) only. A two-sided antagonism in the motility-increasing effect between PPAP and amphetamine and, more pronounced, between PPAP and mazindol was detected. PPAP is substantially less effective in inducing stereotyped behavior than either amphetamine or methamphetamine. PPAP facilitates learning and retention, is highly potent in antagonizing the tetrabenazine-induced depression in behavioral tests and is very effective in the forced swimming test. Whereas amphetamines facilitate performance in a very narrow range of low doses, which turns, at a modest elevation of the dose, into the

opposite effect, PPAP improves performance within a reasonably broad dose range. Based on the peculiar pharmacological profile of PPAP, its potential usefulness in depression, in Alzheimer's disease and in attention-deficit-hyperkinetic disorder seems to be plausible.

Introduction

According to our present classification, there are directly acting sympathomimetics, which stimulate the α- and/or β-adrenoceptors, and indirectly acting sympathomimetics which exert their effect via a stochiometric displacement of the catecholaminergic transmitter. The re-uptake inhibitors, as represented in the classical manner by imipramine, are omitted from the classification as they necessarily influence the serotoninergic system too. However, studies with (−)deprenyl (Knoll, 1978, 1983, 1987) revealed a hitherto unexploited possibility to inhibit the re-uptake of the catecholaminergic transmitters selectively and by a mechanism different from that of the tricyclics.

Monoamine oxidase (MAO) inhibitors potentiate the effect of indirectly acting amines, like tyramine. Due to the potentiation of the effects of tyramine in foodstuffs, sensitive patients treated with MAO inhibitors developed serious, sometimes fatal, hypertensive crises ("cheese effect") which restricted their medical use. (−)Deprenyl challenged this situation, being the first and still the only MAO inhibitor free of the cheese effect.

(−)Deprenyl is handled by the catecholaminergic re-uptake machinery like phenylethylamine (PEA), tyramine, etc. The unique behavior of (−)deprenyl is that, in striking contrast to the indirectly acting amines, it does not displace the transmitter from the storage places, i.e. it is not a releaser. The net result is that (−)deprenyl inhibits the releasing effect of tyramine (Knoll *et al.*, 1968). This most uncommon property for an MAO inhibitor, rendering it safe for medical use, explains why (−)deprenyl, the first selective inhibitor of B-type MAO described in the literature (Knoll and Magyar, 1972), is still the only one in clinical use. The ability of deprenyl to counter the catecholamine-releasing effect of tyramine, PEA, etc., is independent of the MAO inhibitory potency of the drug.

The tyramine-uptake inhibitory property of deprenyl was the starting point of this study. If one gets rid of the MAO inhibitory activity, by changing the propargyl moiety, which binds covalently with the flavin in MAO, to a propyl group, the relation of this new compound to the uptake machinery should remain the same as that of the parent compound. Thus, the way was open for a structure-activity relationship study aiming to select compounds devoid of a catecholamine-releasing property, being,

however, much more potent than deprenyl in interfering selectively with the re-uptake of the catecholamine transmitters. To characterize these new spectrum central nervous system stimulants, operating *de facto* as indirectly acting, nonreleasing sympathomimetics, 1-phenyl-2-propylaminopentane (PPAP) was selected as the reference substance. The detailed pharmacological analysis of PPAP, showing the peculiar pharmacological profile of this new psychostimulant, is the subject of this paper.

Methods

Animals

Experiments were performed on adult CFY (Sprague-Dawley strain) rats, cats and rabbits of both sexes. They were kept in the animal house at a constant temperature (20–21° C) under a standard 12–12 hr light-dark cycle (light on at 6:00 a.m.). Water and food were available *ad libitum*. All the observations were made at comparable times of the day during the light period.

Measurement on the pulmonary artery strip of the rabbit

Strips of the pulmonary artery of the rabbit were prepared and suspended in an organ bath containing 5 ml of Krebs solution, maintained at 37° C and fully aerated with carbogen (5 % CO_2 in oxygen).

We measured tyramine-induced contractions. Cumulative doses (1.3 and 8 μg/ml) of tyramine were used. The test compound was present for 20 min and recovery was checked after washout of the compound.

Measurement on the cat nictitating membrane in vivo

Experiments were carried out in cats, under chloralose-urethane anaesthesia (30 and 200 mg/kg body weight, respectively) and artificial respiration. Blood clotting was prevented by 5 mg/kg of heparin injected at the beginning of the experiment into the femoral vein. Contractions of the nictitating membrane caused by the amines were recorded by an auxotonic writing lever on a kymograph. The amines were injected into the femoral vein.

Measurement of rat's spontaneous motility in the shuttle box

Activity was expressed by the number of crossings through the small gate of the barrier between the two parts of the box.

Simultaneous quantitative analysis of rat's spontaneous and drug-forced (stereotyped) activity patterns

The locomotion, rearing and stereotyped head movements were measured simultaneously by an "Animal Activity Measurement System" as previously described (Timár *et al.*, 1988).

Stereotypy was also assessed by the scoring system (from 0 to 4) of Costall *et al.* (1972).

Measurement of rat's unconditioned escape response with the "modified jumping test"

This test was elaborated for screening psychoactive drugs (Knoll and Knoll, 1964). The excitability of the central nervous system (CNS) is expressed in arbitrary units from 0 to 10.

Measurement of rat's learning and retention in the shuttle box

The acquisition of a two-way-conditioned avoidance reflex (CAR) was analyzed in the shuttle box during 5 consecutive days. The instrument was constructed, according to the device described by Bovet *et al.* (1966), by the Research Institute for Electrical Industry (Budapest, Hungary). It consists of six boxes, each one separated inside by a barrier with a small gate in the middle. Animals were trained to cross the barrier under the influence of a conditioned stimulus (CS) (flash-light). If they failed to do so, they were punished with a footshock (1.3 mA), i.e. an unconditioned stimulus (US). If the rat failed to respond within 5 sec to US, it was noted as escape failure (EF).

The rats were trained with 100 trials per day. One trial consisted of 15 sec intertrial interval, followed by 15 sec CS. The last 5 sec of CS overlapped the 5 sec of US. At each learning session, the number of CAR and intersignal reaction (IR) were automatically counted and evaluated by multi-way analysis of variance (ANOVA).

Measurement of dopamine release from the striatum and tuberculum olfactorium of rats

The method used was the one previously published (Kerecsen *et al.*, 1985).

In this series of experiments, the setup consisted of a Waters pump 510, a Waters V6K injector, a Waters 460 electrochemical detector and a Waters 746 data module (Millipore Corp., Waters Chomatography Division, Milford, Mass., U.S.A.).

Measurement of drug effects in the "forced swimming test"

A variance of the method described by Porsolt *et al.* (1978) was used. The swimming rat stirs the water and these movements are transmitted to a sensitive measuring device which expresses activity in impulses per minute.

Measurement of EEG activity of rats

Male rats, weighing 300–400 g, supplied with chronic cortical electrodes in a plastic capsule, were used. The prepared rat was placed, 30 min after the treatment with the test compound, in a sound and light-proof chamber and EEG activity was measured for 80 min. During the experiment, the animals had free access to food and water. The appearance of the α-, β-, δ- and θ-waves was followed and the changes due to the treatment with test compounds were expressed as a percentage of the ones measured in the saline-treated controls.

Measurement of the dependence capacity of test compounds in rats

The method of Hosoya (1979) for measuring physical dependence of opiates in rats was used. Hosoya's method was based on the observation that the physical dependence, which develops in rats treated daily with opiates, can be detected if the opiate is withdrawn every 7th day of treatment. On the day of withdrawal, the rats are anorectic, thus their body weight is decreasing.

I. Szelényi (personal communication) applied this method with success for detecting the development of physical dependence to amphetamines. He observed that amphetamine-treated rats react to withdrawal with hyperphagia, i.e. their body weight is increasing. We corroborated his findings and applied this method to compare the physical dependence capacity of PPAP to that of amphetamine. Drugs were given daily. Body weight was always measured 4 hr after drug administration. Every 7th day, saline was administered instead of the drug.

Measurement of rat's basal metabolic rate

The basal metabolic rate of rats was determined according to Issekutz and Issekutz (1942). Mobility was dampened by 0.7 g/kg of urethane, i.p. Test compounds were administered after a control measurement lasting 1 hr. Drug-induced changes, expressed as a percentage of the control period, were followed for several hours.

Toxicity

Toxicity was determined in rats, using groups of 10 animals. The LD_{50} was calculated according to Litchfield and Wilcoxon (1949). Deaths occurring within 48 hr were taken into consideration.

Measurement of metabolites of PPAP

Rats of both sexes, weighing 120–150 g, were treated with 20 mg/kg of PPAP, i.p., and their urine was collected for 24 hr. The boiling urine was hydrolyzed in the presence of 1 mol/l HCl for 1 hr and in order to extract the basic constituents the following procedure was carried out: 100 ml of a chloroform-propanol (3:1) mixture was given to the hydrolyzed urine; the pH was adjusted to 10; the mixture was vigorously shaken for 10 min and centrifuged at 1000 *g* for 10 min; the lower phase was extracted by 100 ml HCl of a 1 mol/l concentration, maintaining the pH below 2, and the upper phase was then further treated; 100 ml of a chloroform-propanol (3:1) mixture was given to the water phase; the pH was adjusted to 10; after a 10 min extraction by shaking, the mixture was centrifuged at 1000 *g* for 10 min; the chloroform-propanol mixture was evaporated. The metabolites were identified by GCMS.

Measurement of PPAP uptake into catecholaminergic axon terminals

We performed experiments with [^{14}C]PPAP using a rat whole brain membrane-vesicle suspension, prepared according to Cash and Subbarao (1987) with minor modifications.

Whole brain from male animals, 6 weeks old, was suspended in 60 ml of a Hepes-buffered (pH 7.5) 0.32 M sucrose solution containing 1 mM phenylmethanesulfonylfluoride (PMSF) and the antioxidant, butylated hydroxytoluene (20 μM). The mixture was homogenized with Ultra-Turrax (setting 4) for 2×10 sec. Thereafter, the short procedure (omitting the Ficoll gradient separation) was followed.

Measurement of noradrenaline (NA) and dopamine (DA) uptake in nucleus-free supernatant of rat brain homogenate

Male CFY rat brain cortex or striatum was homogenized in 0.32 M sucrose using a teflon pestle. Nuclei were sedimented by centrifugation at 1000 *g* for 20 min at 4° C. Supernatant was used for the uptake experiments. The incubation medium was oxygen-saturated Krebs-Henseleit buffer of pH 7.0, and after 5 min of preincubation, the uptake process was initiated by adding 5×10^{-8} M [^{3}H]NA or [^{3}H]DA, respectively. The uptake was carried out at 37° C for 5 min and was stopped by dilution

with ice-cold buffer and fast vacuum filtration on GF/B Whatman filter paper. The nonspecific uptake was the amount of [^{3}H]NA or [^{3}H]DA taken up at 37° C in the presence of 10^{-4} M of nisoxetine or 10^{-4} M of benztropine, respectively. The protein concentration was about 0.5 mg/ml.

Displacement studies with catecholaminergic receptors

Male CFY rat brain was homogenized in buffer and crude membrane was prepared by repeated centrifugation (50,000 *g* for 15 min) and washing procedure. In the case of the α_2-receptor, the P_2 preparation was used. The incubation was carried out in 50 nM Tris-HCl buffer of pH 7.4, containing 1 mM $MgCl_2$ for α_2-receptors, and in 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1 % ascorbic acid solution when the DA receptor ligand binding was measured. The incubation was performed at 37° C for 10 min with [^{3}H]spiroperidol and at 25° C for 25 min with adrenergic receptor ligands. The binding procedure was terminated by rapid vacuum filtration on GF/B Whatman filter paper.

Drugs used

The phenylalkylamines, as well as [^{14}C]PPAP (specific activity: 15 mCi/nmol) were synthesized by Chinoin, Budapest, Hungary. Compounds used were as follows: tyramine HCl (Serva Feinbiochemica, Heidelberg, Germany), β-phenylethylamine HCl, (+)amphetamine sulphate, methamphetamine HCl and methylphenidate HCl (all from Sigma Chemical Co., St Louis, MO, U.S.A.), mazindol (Alkaloida, Tiszavasvári, Hungary), phenmetrazine (EGIS, Budapest, Hungary), amfepramon HCl (Merrell-Toraude Laboratoires de Développement, France), chlorphentermine HCl (EGIS), tetrabenazine HCl (synthesized by Prof. C. Szántay, Department of Organic Chemistry, University of Technical Sciences, Budapest, Hungary), α-methylparatyrosine methylester HCl (Sigma), desmethylimipramine HCl and (−)deprenyl HCl (Chinoin), codeinum HCl (Alkaloida), prazosine HCl (Biogal, Debrecen, Hungary), benztropin methansulfonate (Sigma), nisoxetine (Eli Lilly & Co, Indianapolis, IN, U.S.A.), (+)butaclamol (Ayerst Labs., New York, NY, U.S.A.), clonidine HCl (Boehringer, Ingelheim am Rhein, Germany), [^{3}H]noradrenaline, [^{3}H]dopamine, [^{3}H]dihydroalprenolol, [^{3}H]WB-4101, [^{3}H]clonidine and [^{3}H]spiroperidol (Amersham, Buckinghamshire, England).

Statistical analysis

Depending on the need, either the two-tailed Student's *t*-test, or the Mann-Whitney U-test or the multi-way analysis of variance (ANOVA) was

used. Mean values are presented $\pm$ S.E.M. A p value of 0.05 or less was considered to be significant.

Results

Pharmacological analysis of the effects of PPAP

Structure-activity relationships regarding the tyramine uptake inhibitory effect of new phenylalkylamine derivatives in the pulmonary artery strip of the rabbit

Deprenyl inhibits the uptake of tyramine in the pulmonary artery strip of the rabbit (Knoll, 1987). This effect, however, is relatively weak and the ID_{50} cannot be established. We got rid of the MAO inhibitory activity of deprenyl by changing the propargyl group to a propyl group. No change in tyramine uptake inhibitory potency was observed. The ethyl analogue of this substance, however, inhibited tyramine uptake in a dose-dependent manner. Methamphetamine, one of the best-known highly active releasers of catecholamines, inhibits the uptake of tyramine with considerable potency ($ID_{50} = 8.03 \times 10^{-6}$ M). We were interested in developing such analogues which are more potent than methamphetamine in inhibiting the uptake of tyramine, but which are, like deprenyl, devoid of the catecholamine-releasing property.

Structure-activity relationship studies revealed that those methamphetamine analogues which are properly substituted at both the nitrogen moiety and the *a*-carbon lose the releasing property. Out of 20 compounds synthesized for the structure-activity relationship study, PPAP, which inhibited tyramine uptake in the pulmonary artery strip of the rabbit with an $ID_{50} = 1.22 \times 10^{-6}$ M, proved to be one of the best representatives of the desired spectrum. We selected it as the reference substance for demonstrating the pharmacological profile of those new phenylalkylamine derivatives which are indirectly acting sympathomimetics without possessing the catecholamine-releasing property.

In vivo demonstration that, in contrast to the known psychostimulants, PPAP is free of the noradrenaline-releasing property and antagonizes the effect of the releasers

The cat nictitating membrane is a highly sensitive detector for demonstrating the *in vivo* catecholamine-releasing effect of amphetamine and analogues. In contrast to the transient nature of the noradrenaline-releasing effect of PEA, amphetamine has a long-lasting effect. Methylamphetamine,

as well as the clinically used stimulants, methylphenidate, phenmetrazine, chlorphentermine and mazindol, which we used as examples, proved to be potent noradrenaline releasers *in vivo* in the cat nictitating membrane test. In striking contrast to the known psychostimulants, PPAP was not only devoid of the noradrenaline-releasing property, but, as expected from the *ex vivo* experiments with tyramine, it inhibited dose-dependently the effect of PEA.

The motility-increasing effect of PPAP

PPAP starts to increase motility significantly from 2 mg/kg on and inhibits it in a very high dose (50 mg/kg) only. Amphetamine was much more potent than PPAP in the test. PPAP, in a dose of 1 mg/kg, which per se does not increase motility significantly, completely antagonized the tetrabenzine (1 mg/kg)-induced depression of motility. Table I shows that amphetamine and PPAP, administered together, antagonize each others motility-enhancing effect. The two-sided antagonism was even more pronounced with the combination of mazindol and PPAP (see Table I).

Quantitative analysis of the rat's spontaneous and drug-forced (stereotyped) activity patterns in PPAP-treated rats

PPAP and amphetamine-induced dose-related changes in the spontaneous activity of rats were quantitatively analyzed by means of a com-

TABLE I

Influence on motility of PPAP in combination with amphetamine and mazindol, respectively

Compound	Dose (mg/kg)	Compound	Dose (mg/kg)	Number of crossings (during 30 min)
1. Saline	–	Saline	–	11.8 ± 3.1
2. Amphetamine	2	Saline	–	77.8 ± 18.7** 2:1
3. Mazindol	5	Saline	–	72.4 ± 17.3** 3:1
4. Methylphenidate	5	Saline	–	54.0 ± 15.3** 4:1
5. PPAP	5	Saline	–	56.8 ± 10.8*** 5:1
6. Amphetamine	2	PPAP	5	92.3 ± 20.3
7. Mazindol	5	PPAP	5	22.7 ± 6.9* 7:3
8. PPAP	5	Amphetamine	2	58.9 ± 11.9
9. PPAP	5	Mazindol	5	28.2 ± 9.5* 9:5

Groups of 10 rats (5 males and 5 females).

Drugs were injected s.c. The second injection was given 30 min after the first injection. Motility was measured after the 2nd injection following a 30 min adaptation period in the shuttle box.

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$: Student's *t*-test for two means.

puterized system. The parameters measured were: duration of the resting state (min), duration of spontaneous motility (min), the distance covered (cm), time spent with rearing (min), the number of rearings, time spent with stereotyped head movements (STHMV) (min) and their number. Amphetamine was administered in small doses (0.5 and 1 mg/kg), which are stimulatory, and in high doses (5 and 10 mg/kg), which are inhibitory in learning tests. The resting time decreased significantly with all doses. Small doses of amphetamine increased spontaneous motility patterns characteristically, the maximum effect was reached with the 5 mg/kg dose. With the highest dose (10 mg/kg), the well-known inhibitory effect of amphetamine (Knoll and Knoll, 1975) was observed, 10 mg/kg amphetamine being much less potent than 1 mg/kg in increasing motility. Rearings and STHMV changed significantly with the highest amphetamine dose only. PPAP was given in 1, 5, 10 and 20 mg/kg doses, all of which proved to be stimulatory in learning tests. The resting state decreased and the time spent with movements increased significantly with 5 mg/kg PPAP. The effect of the 10 mg/kg dose did not differ significantly from that of the smaller one. The highest dose given (20 mg/kg) was more potent than the smaller ones, no sign of inhibition was observed. The time and number of rearings increased significantly with the 10 mg/kg dose only and the number of STHMV increased significantly with the 20 mg/kg dose.

In a series of experiments, stereotyped behavior was measured by direct observation at 10 min intervals after the injection of PPAP and reference substances. For evaluation, the scores introduced by Costall *et al.* (1972) were used. The dose-dependent increase in the scores with maximums over 3 was found with amphetamine, methamphetamine and methylphenidate. PPAP, in doses of 5, 10 and 25 mg/kg, was substantially less effective than either amphetamine or methamphetamine.

Comparison of the effects of PPAP and amphetamine in a one-way escape test

Using the modified jumping test (Knoll and Knoll, 1964), we compared the inhibitory effect of high doses of amphetamine and PPAP on performance. A dose as high as 50 mg/kg PPAP started to lower the ability of the rats to escape from the deleterious circumstances and this effect was of short duration. Amphetamine (5 and 7.5 mg/kg) inhibited escape completely and the effect was long-lasting. PPAP antagonized in a dose-dependent manner the inhibitory effect of amphetamine.

Comparison of the effects of PPAP and amphetamine on learning and retention of rats in the shuttle box

Amphetamine improved within a small dose range the performance of rats in behavioral tests. At subcutaneous administration, the optimum dose

range for amphetamine was between 0.5 and 2.5 mg/kg. Higher doses deteriorate performances (Knoll and Knoll, 1975).

PPAP and its close structural relatives enhance, from low to relatively high doses, learning and retention in behavioral tests. PPAP, in doses from 2 to 15 mg/kg, significantly improved the acquisition of conditioned avoidance responses (CARs) in a dose-dependent manner. Only very high doses (over 30 mg/kg) started to inhibit performance. As an example, Fig. 1 shows the beneficial effect of 5 mg/kg PPAP on learning and retention in the shuttle box. It is evident from this Figure that, under the influence of PPAP treatment, the percentage of CARs increased to a very high level during the 5 consecutive days of training. Whereas in the saline-treated group a substantial number of escape failures (EFs) could be observed, none of the PPAP-treated animals failed to respond to the unconditioned stimulus.

There was a significant increase in the number of intersignal reactions during PPAP treatment, indicating the state of a general excitation of the

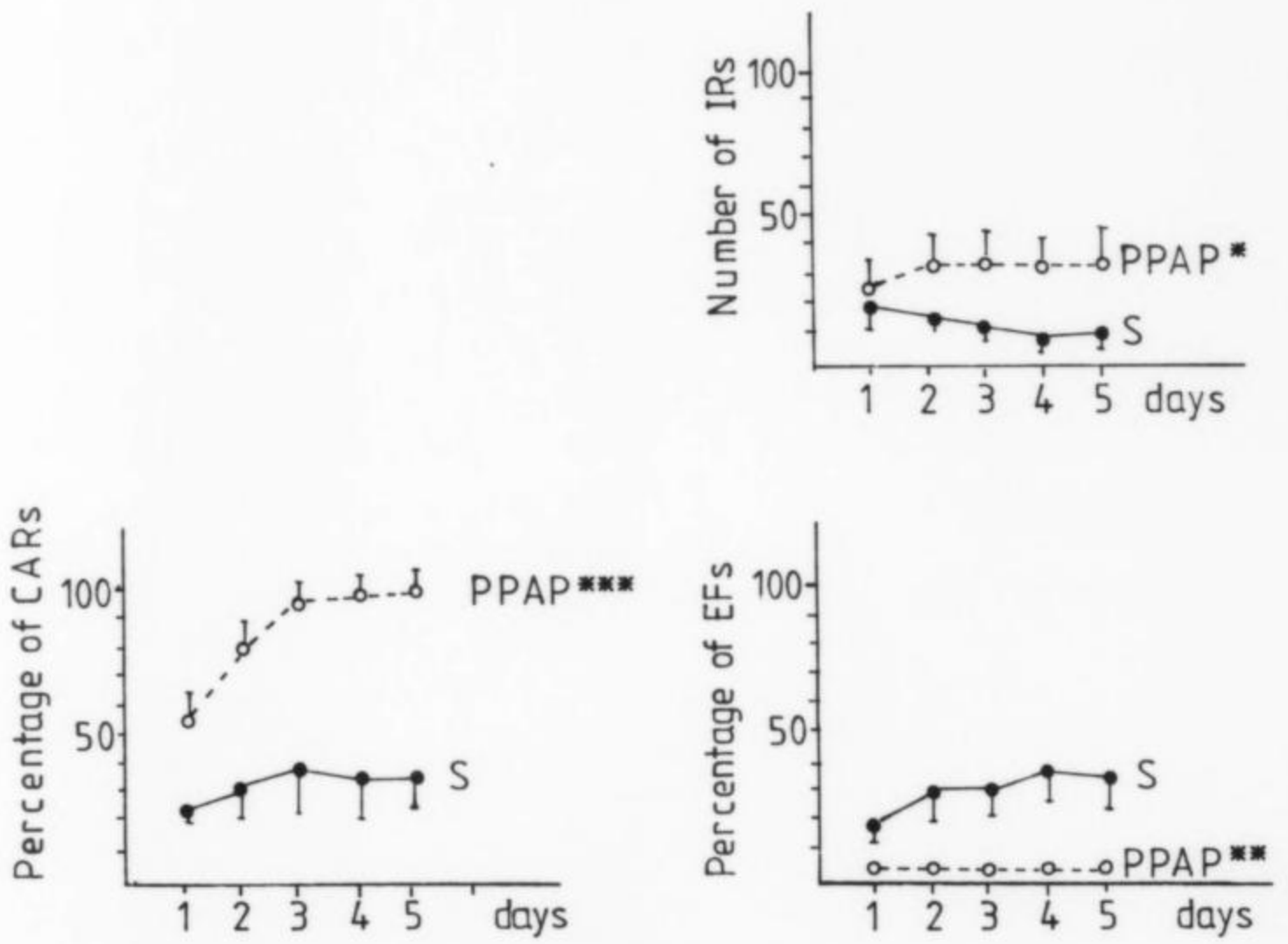

FIG. 1

Enhancement of learning and retention of rats in the shuttle box by daily treatment with PPAP.

S: Saline; PPAP: 1-phenyl-2-propylaminopentane; CAR: conditioned avoidance response; EF: escape failure; IR: intersignal reaction.

S or PPAP (5 mg/kg, s.c.) were administered daily 30 min prior to measurements. Rats were trained at 100 trials daily. $N = 10$ (5 males, 5 females).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: Significant to saline control (ANOVA).

rats. Considering the excellent performance of the rats in the test, the intensity of the intersignal activity was acceptable. In the case of amphetamine, the intersignal activity is very high compared to the improvement in performances.

PPAP was within a broad dose range (1–15 mg/kg) very efficient in countering the tetrabenazine-induced depression, whereas amphetamine and methamphetamine were effective in a narrow range only (0.5–2 mg/kg). Figure 2 demonstrates that 10 mg/kg of PPAP is completely erasing the effect of tetrabenazine.

Pretreatment of rats with 60 mg/kg of α-methylparatyrosine (α-MT) 4 hr before PPAP treatment did not significantly change the effect of either 5 mg/kg or 15 mg/kg of PPAP in the shuttle box, whereas this pretreatment completely antagonized the effect of 2 mg/kg of amphetamine, which was found to be the optimal dose for improving performance.

Comparison of the effects of amphetamine and PPAP on the release of dopamine from the striatum and tuberculum olfactorium of rats

Table II shows the time-related decline of the amount of dopamine released from the isolated striatum of rats during 3 consecutive 20 min

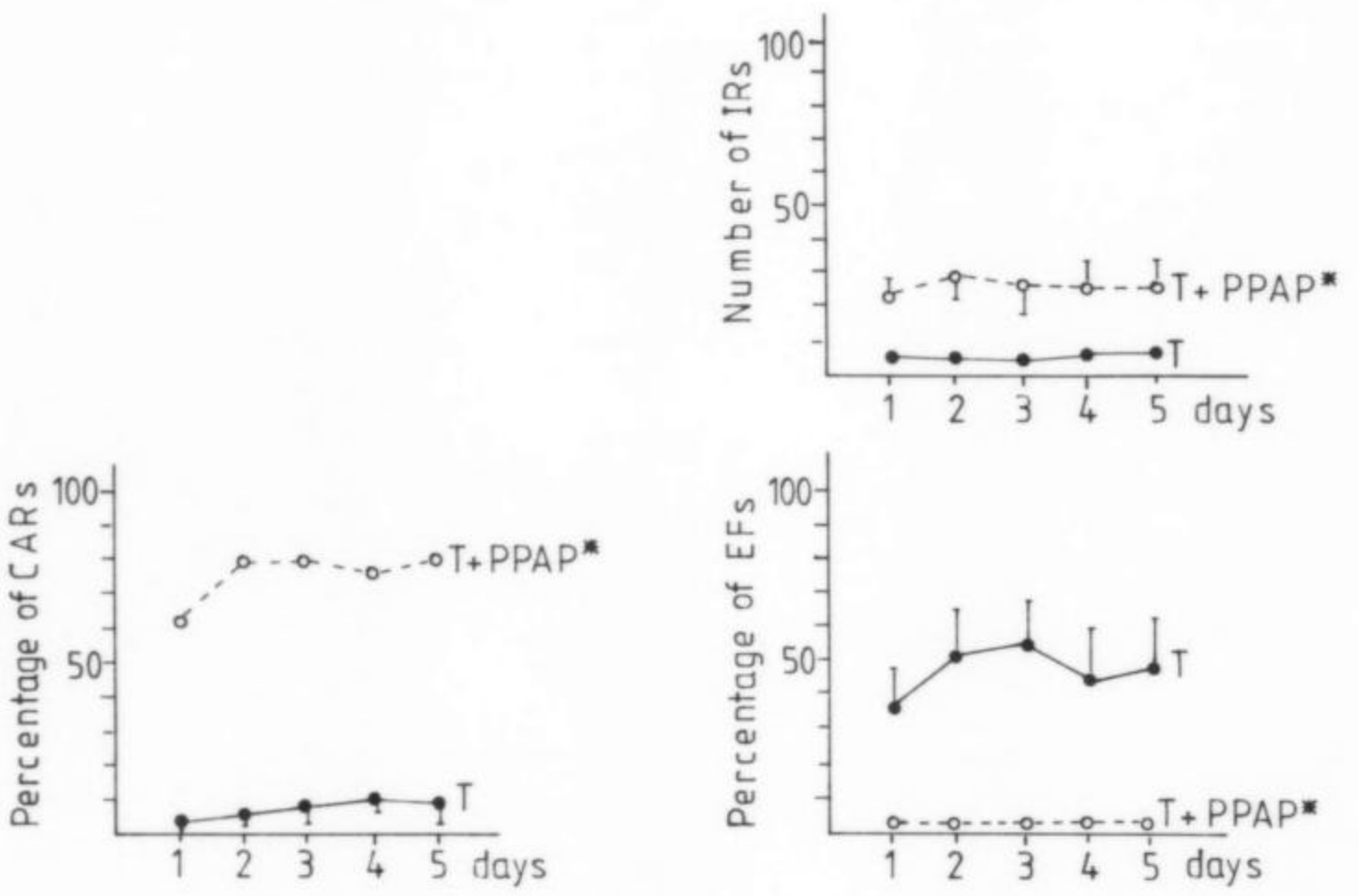

FIG. 2

Antagonistic effect of PPAP in rats against tetrabenazine (T)-induced depression of learning in the shuttle box.

T (0.5 mg/kg) + PPAP (10 mg/kg) or T + saline were administered s.c. 30 min prior to measurement. N = 10 (5 males, 5 females). For details see Fig. 1.

* $p < 0.001$: Significant to tetrabenazine + saline (ANOVA).

periods. If after a 20 min bathing period (control) the bath fluid was changed to one containing 5 μg/ml of amphetamine, the amount of dopamine released from the axon terminals during the second 20 min period was significantly higher than before treatment, due to the dopamine-releasing effect of amphetamine. During the third 20 min period, after washing out the drug, a sharp decline in the amount of dopamine released from the tissue was found.

Table III shows that amphetamine acts similarly on the tuberculum olfactorium, one of the representatives of the mesolimbic dopaminergic system. Tables II and III further demonstrate that 5–20 μg/ml of PPAP left the amounts of dopamine released from the rat striatum and tuberculum

TABLE II

Effect of PPAP, amphetamine and their combination on the release of dopamine from the striatum of rats

Compounds	Amount of dopamine released from the tissue (nmol/g wet tissue ± S.E.M.; n = 4)			
	20 min incubation periods			
	1st (control)	2nd (drug-treated)	3rd (washing)	Significance* (p)
	a	b	c	
Saline	2.25 ± 0.21	1.34 ± 0.18	1.07 ± 0.22	a:b < 0.01 a:c < 0.01 b:c > 0.05
Amphetamine 5 μg/ml	2.02 ± 0.19	3.62 ± 0.28	1.62 ± 0.16	a:b < 0.01 a:c < 0.05 b:c < 0.01
PPAP 5 μg/ml	2.31 ± 0.20	1.38 ± 0.15	1.03 ± 0.11	a:b < 0.01 a:c < 0.01 b:c > 0.05
PPAP 10 μg/ml	2.38 ± 0.22	1.60 ± 0.20	0.79 ± 0.09	a:b < 0.02 a:c < 0.01 b:c < 0.05
PPAP 20 μg/ml	1.99 ± 0.17	1.01 ± 0.13	0.90 ± 0.12	a:b < 0.01 a:c < 0.01 b:c > 0.05
Amphetamine 5 μg/ml + PPAP 20 μg/ml	2.22 ± 0.18	2.01 ± 0.19	0.81 ± 0.09	a:b > 0.05 a:c < 0.01 b:c < 0.01

* Student's *t*-test for two means.

TABLE III

Effect of PPAP, amphetamine and their combination on the release of dopamine from the tuberculum olfactorium of rats

Compound	Amount of dopamine released from the tissue (nmol/g wet tissue ± S.E.M.; n = 4)			
	20 min incubation periods			
	1st (control)	2nd (drug-treated)	3rd (washing)	Significance*
	a	b	c	
Saline	2.34 ± 0.18	1.88 ± 0.18	1.32 ± 0.13	a:b > 0.05 a:c < 0.01 b:c < 0.05
Amphetamine 5 μg/ml	2.28 ± 0.16	3.59 ± 0.27	2.21 ± 0.18	a:b < 0.01 a:c > 0.05 b:c < 0.01
PPAP 5 μg/ml	2.51 ± 0.22	2.02 ± 0.17	1.03 ± 0.11	a:b > 0.05 a:c < 0.01 b:c < 0.05
PPAP 10 μg/ml	2.40 ± 0.19	2.03 ± 0.16	1.12 ± 0.13	a:b > 0.05 a:c < 0.01 b:c < 0.05
PPAP 20 μg/ml	1.99 ± 0.18	1.52 ± 0.11	1.03 ± 0.11	a:b < 0.05 a:c < 0.01 b:c < 0.05
Amphetamine 5 μg/ml + PPAP 20 μg/ml	2.31 ± 0.19	2.38 ± 0.20	0.68 ± 0.11	a:b > 0.05 a:c < 0.001 b:c < 0.001

* Student's *t*-test for two means.

olfactorium unchanged. PPAP inhibited the dopamine-releasing effect of amphetamine in the striatum (Table II), as well as in the tuberculum olfactorium (Table III), because it interferes with the uptake of amphetamine into the axon terminals and into the vesicles.

The antidepressant effect of PPAP in the Porsolt test

The forced swimming test (Porsolt *et al.*, 1978) is used as a screening method for predicting the antidepressant effect of drugs. We measured the effect of 4, 8 and 16 mg/kg of PPAP in this test by injecting subcutaneously the compounds for 5 consecutive days and measured the rat's performance in the swimming test 30 min after administration of the 5th dose. We also

checked the effect of single doses of 4, 8 and 16 mg/kg of PPAP. Figure 3 shows the dose-related effect of single doses of PPAP on the performance of rats. The facilitation of the swimming activity of the rats by the drug was highly significant even with the lowest dose given (4 mg/kg). Essentially the same effect was found in rats treated with PPAP for 5 consecutive days. Amphetamine (2–8 mg/kg) was not convincingly efficient, (−)deprenyl (2–16 mg/kg) was completely ineffective in this test.

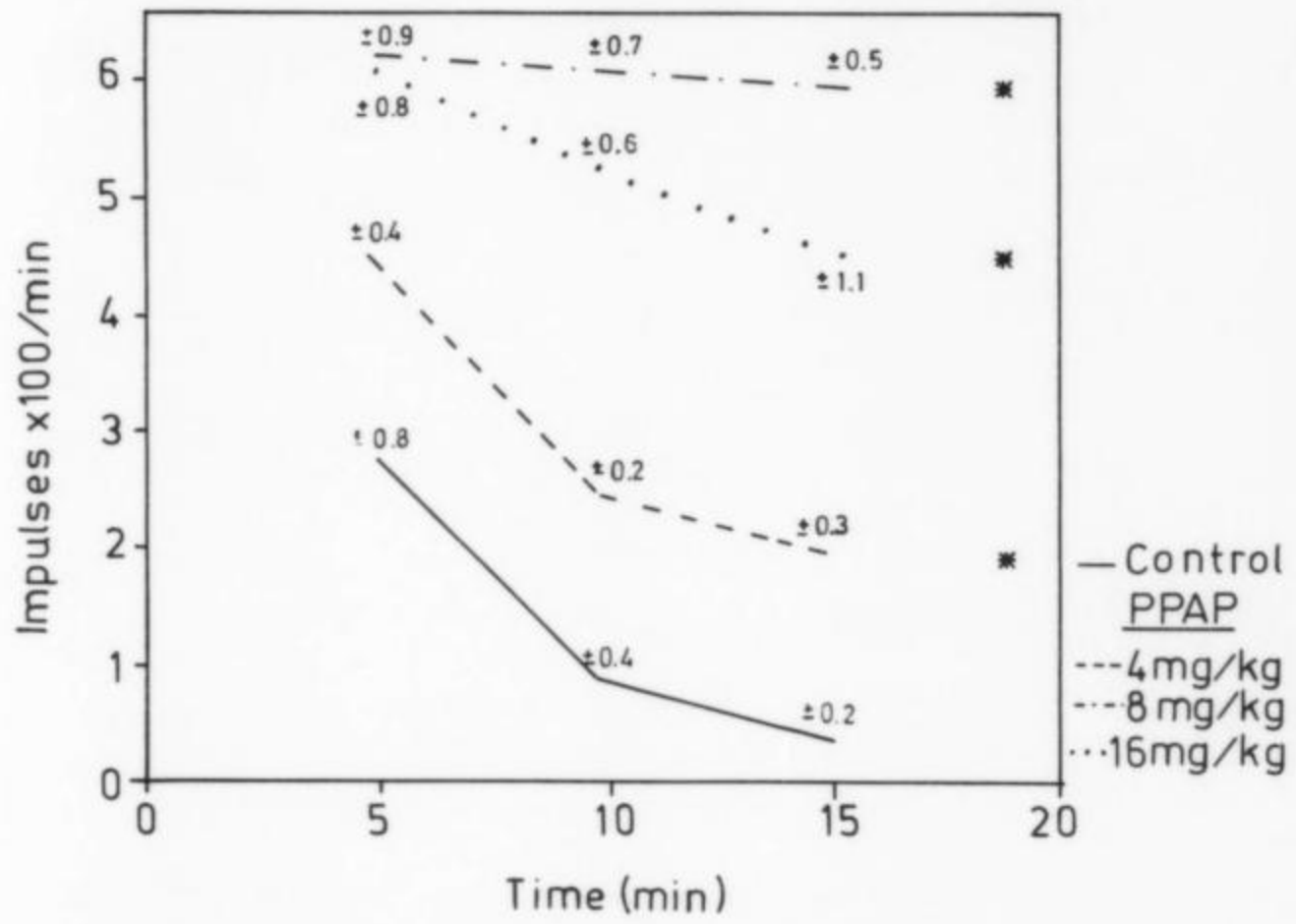

FIG. 3

Dose-related antidepressant effect of PPAP in the forced swimming test in rats. PPAP was given s.c. 30 min prior to measurement. N = 12.
*: Statistically significant (Student's *t*-test for two means).

Comparison of the effects of PPAP and amphetamine on the EEG activity of rats.

Amphetamine and PPAP influenced the brain electric activity differently. The most pronounced difference was found in the drug-induced changes of θ-activity as shown in Fig. 4, further illustrating the differences in the mechanism of action by which these two psychostimulatory sympathomimetics act in the brain.

Trial to test the dependence capacity of PPAP in rats

We compared in an 8-week experiment in rats the dependence capacity of codeine (100 mg/kg, orally), amphetamine (2.5 mg/kg, s.c.) and PPAP

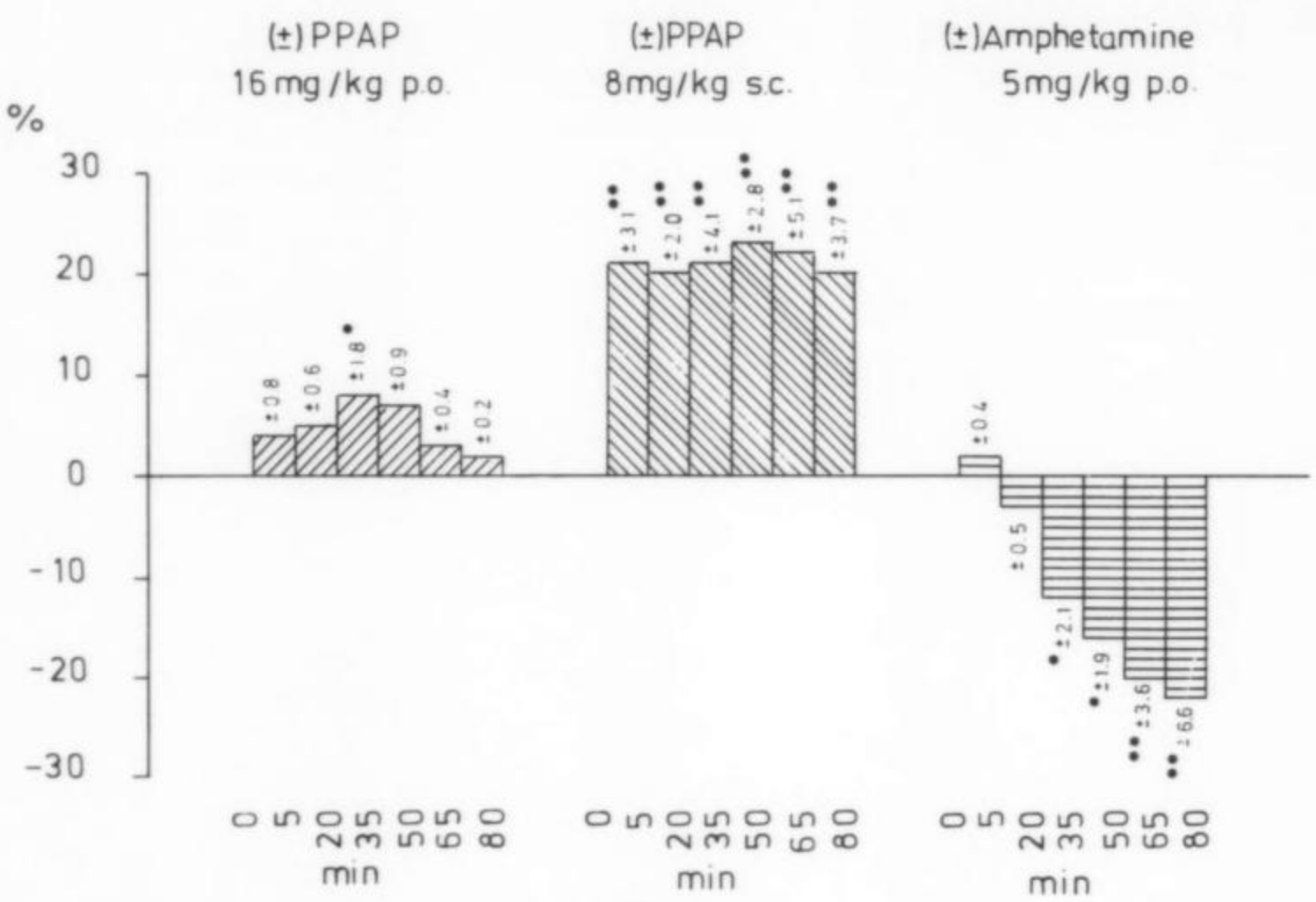

FIG. 4

Influence of PPAP and amphetamine on the θ-activity of the rat cortex. N = 6.
* $p < 0.05$, ** $p < 0.01$: EEG frequency changes after administration of drugs differ significantly from the control value of the untreated rats (Student's *t*-test for paired samples).

(10 mg/kg, s.c. and 12 mg/kg, orally). Drugs were administered daily for 6 consecutive days, followed by a day of withdrawal. The mean change in body weight over 8 weeks is shown in Fig. 5. Physical dependence developed in the groups of rats treated with either codeine or amphetamine. However, a very slight change only was observed in the group of rats treated s.c. with 10 mg/kg of PPAP. As the s.c. doses of amphetamine and PPAP used in this experiment can be taken as equally active ones in improving the performance of rats in behavioral tests, the dependence capacity of PPAP in comparison to that of amphetamine seems to be reasonably low.

Comparison of the effect of PPAP and amphetamine on the basal metabolic rate of rats

Amphetamine and methylamphetamine increase the oxygen consumption of rats in a dose-dependent manner. The effect of the amphetamines is long-lasting. As an example, 2.5 mg/kg of amphetamine, given s.c., increased oxygen consumption for several hours. PPAP (10 mg/kg) induced a moderate increase in oxygen consumption and the effect lasted

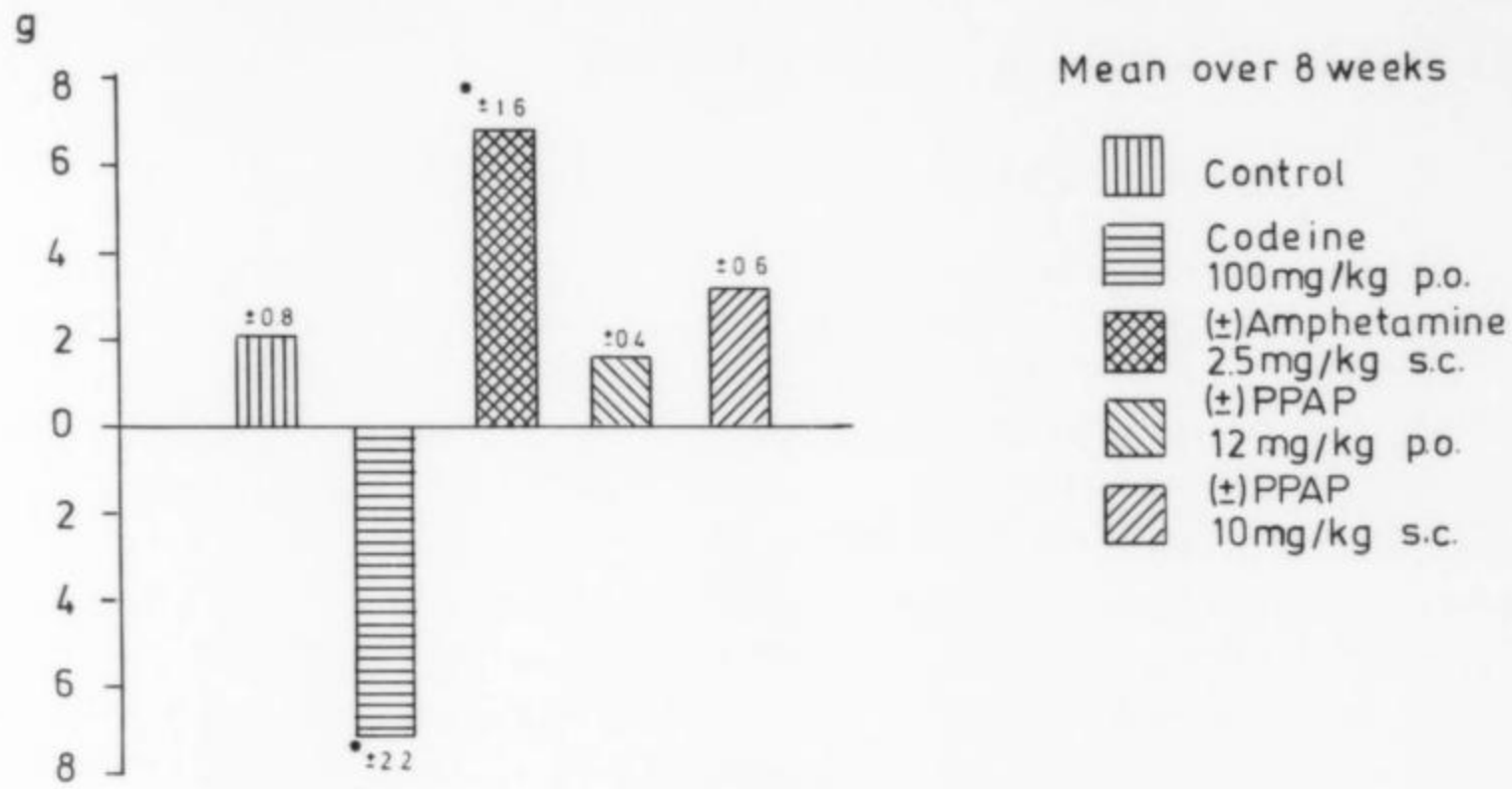

FIG. 5

Comparison of the dependence capacity of codeine, amphetamine and PPAP in the Hosoya test as assessed by the change in body weight.

Rats were treated with the drugs daily for 8 weeks, except on the days of withdrawal. $N = 6$.

* $p < 0.01$: Changes in body weight after withdrawal of drugs differ significantly from the control value of the untreated rats (Student's *t*-test for nonpaired samples).

about 1 hr. This means that a dose of PPAP, which exerts a maximal effect in the shuttle box, is only slightly changing the basal metabolic rate of the rats.

Toxicity of PPAP in comparison to amphetamine and methylamphetamine

PPAP (ID_{50} = i.v. 27 mg/kg; s.c. 110 mg/kg; p.os 270 mg/kg) was found to be equally toxic orally and slightly more toxic parenterally than its parent compound, methylamphetamine, when tested in a parallel experiment.

Metabolism of PPAP

Standard PPAP gave two peaks: 91 (cycloheptatrien cation from the benzyl part) and 114 (the propylamine side chain). The GC retention time was 9.73 min. In the urine, unchanged PPAP and 2 main metabolites were found. The loss of the propyl group was shown by a peak of 72; i.e. 42 was lost from 114. The GC retention time of this peak was 5.28 min (metabolite I). An addition of 16 to the peak of 91, i.e. a peak of 107 was also detected, speaking in favor of the para-hydroxylation of the aromatic ring. The GC retention time of this peak was 9 min (metabolite II). The para-hydroxylation of metabolite I was negligible. All in all, about 60 % of unchanged PPAP, 20 % of metabolite I and 20 % of metabolite II was detected.

Biochemical analysis of the effects of PPAP

The uptake of [^{14}C]PPAP into the catecholaminergic neurons

The pharmacological analysis of PPAP clearly indicated that this phenylethylamine derivative is taken up by catecholaminergic axon terminals. The uptake through the vesicle membrane is ATP-dependent (Iversen, 1967). Considering the specific activity of our substance, we measured the uptake of [^{14}C]PPAP into the membrane vesicles by giving 3 μM of the labelled substance to 0.5 mg/ml of protein (Lowry *et al.*, 1951). Under these circumstances, the uptake reached saturation within 1 min. The ratio between the amount of [^{14}C]PPAP at 4° C and 37° C was 0.55 within 1 min and was near 1 within 10 min, indicating that by reducing the time given for the uptake, the temperature-dependent uptake has a greater share and 70 % of the uptake in 1 min at 37° C was inhibited by 1 mM of desmethylimipramine (DMI). The DMI-sensitive uptake for [^{14}C]PPAP (specific uptake) was found to be 39.5 mM/min/mg protein and independent from the presence or absence of ATP (1 mM). The specific uptake of [^{14}C]PPAP was decreased by PPAP, as well as by DMI, in a concentration-dependent manner. The inhibition of the specific uptake of [^{14}C]PPAP, however, seems to be complex in nature, being influenced by ATP. In the presence of ATP, the "gross" IC_{50} value for DMI was found to be about 3 μM and that for PPAP about 7 μM.

Inhibition of the uptake of [^{14}C]noradrenaline and [^{3}H]dopamine by PPAP in the nucleus-free supernatant of the rat cortex homogenate

PPAP inhibited the uptake of noradrenaline in the rat cortex homogenate and surpassed the uptake inhibitory effect of (−)deprenyl considerably (for PPAP $IC_{50} = 5 \times 10^{-8}$ M; for (−)deprenyl $= 7 \times 10^{-6}$ M).

PPAP inhibited the high affinity uptake of dopamine in the rat striatum and was as potent in this respect as benztropin, which was used as the reference substance.

Analysis of the inhibition of the uptake of [^{3}H]noradrenaline by PPAP in a purified synaptosomal fraction and in a membrane-vesicle suspension prepared from rat brain

We measured the inhibition of the uptake of [^{3}H]NA by PPAP and DMI in a membrane-vesicle suspension prepared from the cortex and whole brain of the rat and in a purified synaptosomal fraction prepared from the whole brain. Table IV shows that the membrane-vesicle preparation, which can be prepared faster and more easily than the purified synap-

TABLE IV

Inhibition of [^{3}H]noradrenaline ([^{3}H]NA) uptake by PPAP and DMI in a membrane-vesicle suspension and in a purified synaptosomal fraction prepared from rat brain

Preparation	Temp. (°C)	NA (M)	[^{3}H]NA (dpm/ml)	Specific [^{3}H]NA uptake (°)(%)	IC_{50} (M) DMI	PPAP
Membrane-vesicle from cortex (a)	37	5.10^{-8}	2.10^5	25	$<10^{-6}$	$<10^{-6}$
Membrane-vesicle from whole brain (a)	37	10^{-7}	5.10^5	75	$<10^{-8}$	$\sim10^{-8}$
Purified synaptosome from whole brain (b)	22	10^{-7}	5.10^5	68	no inhibition	
Purified synaptosome from whole brain (b)	37	10^{-7}	5.10^5	79	$<10^{-8}$	$\sim10^{-8}$

(a) Cash and Subbarao (1987): see paragraph "The uptake of [^{14}C]PPAP into the catecholaminergic terminals".

(b) Hajós (1975).

(c) Assay conditions: Hepes-buffered salt solution (Cash and Subbarao, 1987) in the presence of 1 mM of ascorbic acid; 1 mg protein/ml; uptake time = 5 min; filtration through Whatman 6F/B glass fiber filter; washing: 2 × 3 ml of ice-cold Hepes-buffered salt solution containing 0.1 mM DMI; nonspecific uptake was determined in the presence of 0.1 mM NA.

tosomal fraction, is as sensitive to the inhibitors as the other one. We, therefore, primarily used the membrane-vesicle preparation. The data in Table IV also show that the PPAP- and DMI-induced inhibition of the uptake is temperature-dependent and that the estimated IC_{50} values for DMI and PPAP are $<10^{-8}$ and $\simeq 10^{-8}$, respectively. Table V shows that the PPAP-induced inhibition of [^{3}H]NA uptake is increasing with time, reaching the maximum effect (70 %) within 10 min. The presence of ATP increases the effect, whereas in an isoosmotic buffer (Hepes) containing only sodium ions, no inhibition by PPAP could be detected.

TABLE V

ATP dependence of the inhibition of [^{3}H]noradrenaline ([^{3}H]NA) uptake ([a]) in rat whole brain membrane vesicles by PPAP

Experimental conditions	[^{3}H]NA uptake (dpm/ml)		PPAP (10^{-4} M)	Inhibition of specific uptake (%)
	Total	Nonspecific		
1 min	12797 ± 832	6244 ± 98	11127 ± 1118	25
5 min	26427 ± 440	6913 ± 414	15451 ± 127	56
10 min	35158 ± 178	8473 ± 171	16552 ± 165	70
5 min – ATP ([b])	17030 ± 112	5891 ± 477	7430 ± 9	86
5 min – Na^+ ([c])	16206 ± 155	6246 ± 502	16031 ± 815	2

([a]) For experimental details see Table IV.
([b]) In the presence of 1 mM ATP.
([c]) In the presence of an isoosmotic buffer (Hepes) containing only sodium ions.

Figure 6 shows the concentration dependence of the PPAP- and DMI-induced inhibition of [^{3}H]NA uptake in the presence of ATP. The curves indicate differences in the mechanism of action of the two inhibitors. In the membrane of the axon terminal, noradrenaline is known to bind with high affinity to the carrier protein. This process is temperature- and Na^+-dependent (uptake 1a) (Iversen, 1967). On the other hand, noradrenaline is taken up by the membrane of the storage vesicles (uptake 1b) and this is an ATP-dependent function. We can calculate from the curves two IC_{50} values (1 and 2) for both inhibitors: 7 nM and 1 μM for PPAP and 1 nM and 16 μM for DMI.

Considering the effects of temperature and ATP on the inhibition of [^{3}H]NA uptake by PPAP and DMI, the IC_{50} (1) values may be related to uptake 1a, and the IC_{50} (2) values to uptake 1b. This would be in agreement with the known fact that DMI is a weak inhibitor of the uptake of noradrenaline through the membrane of the vesicles. Thus, PPAP is considered to be a much more potent inhibitor of uptake 1b than DMI.

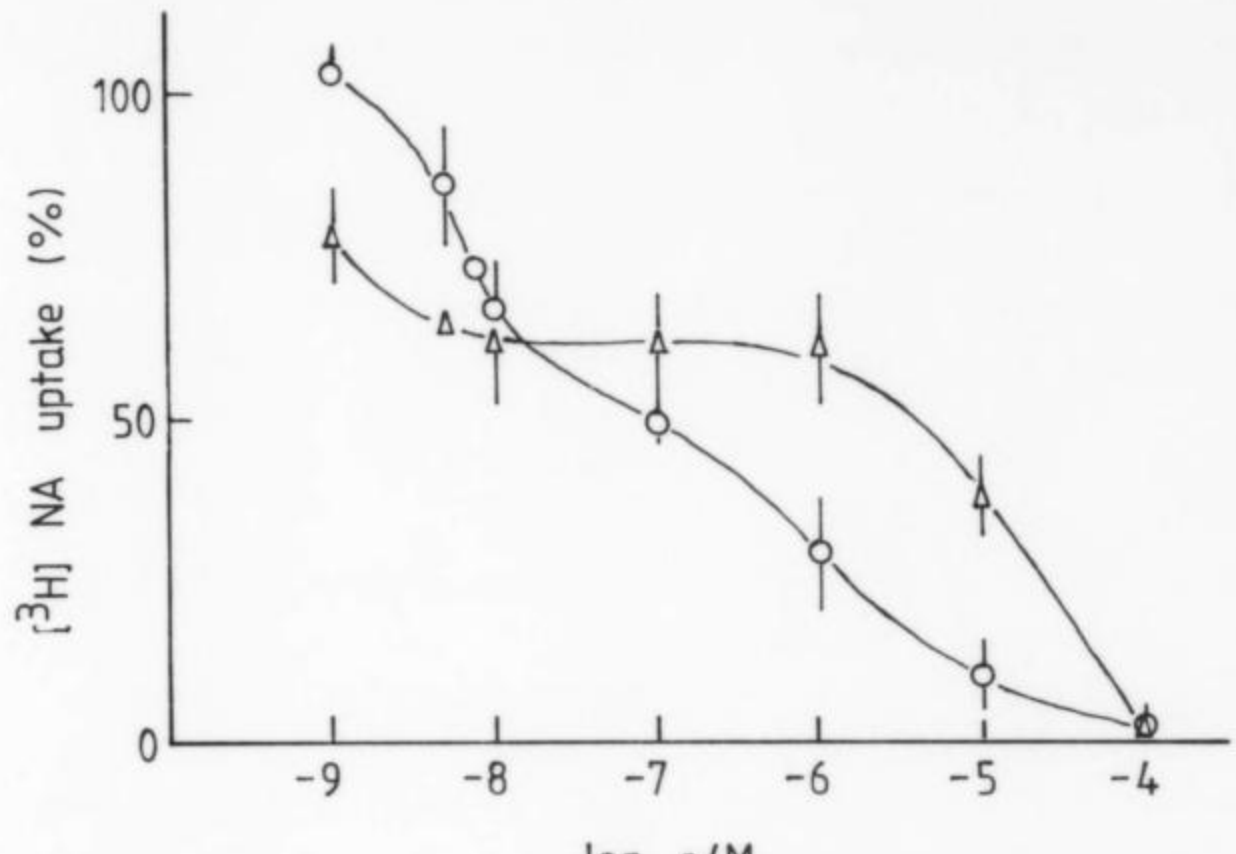

FIG. 6

Effects of PPAP (○) and desmethylimipramine (△) on the specific uptake of [^{3}H]noradrenaline by rat whole brain membrane vesicles at 37° C in the presence of 1 mM ATP. N = 4.

Assay conditions: 0.1 μM [^{3}H]NA; time: 10 min; for other details see Table IV.

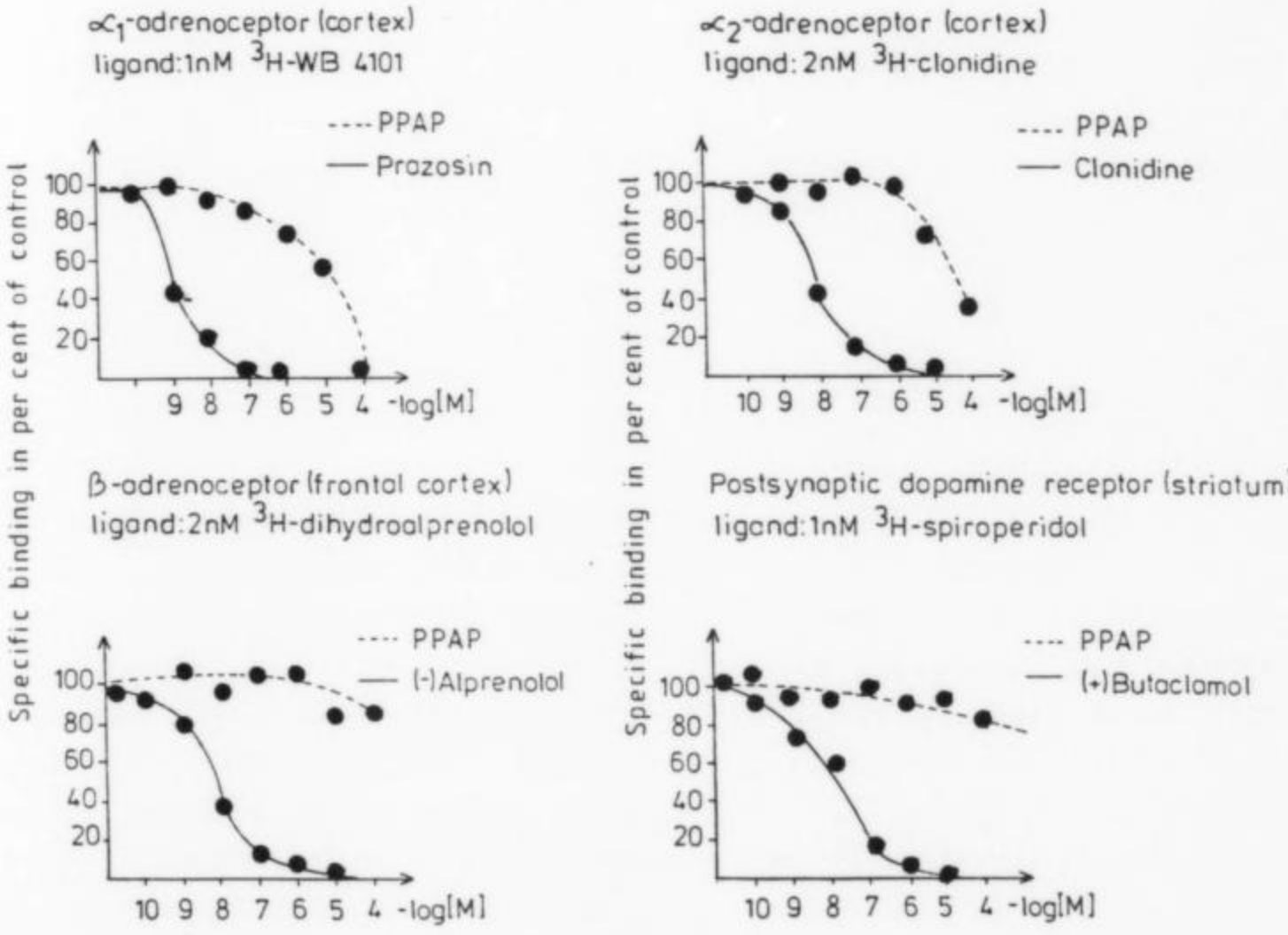

FIG. 7

Ineffectiveness of PPAP in displacing specific ligands to α_1-, α_2- and β-adrenoceptors and to postsynaptic dopamine receptors.

Analysis of the affinity of the new phenylethylamines to catecholaminergic receptors

Displacement studies were performed on rat brain membrane preparations. Figure 7 shows that PPAP was ineffective within a reasonable concentration range in displacing the specific ligands of α_1-, α_2- and β-adrenoceptors as well as of postsynaptic dopamine receptors.

Discussion

PPAP, a new spectrum psychostimulant described in this paper, is taken up by the catecholamine axon terminal membrane and the vesicular membrane, and thus interferes selectively with the uptake of catecholamines and indirectly acting releasers, like tyramine; it is, however, free of the catecholamine-releasing property. This highly individual pharmacological profile of PPAP was demonstrated by a pattern of pharmacological and biochemical methods.

Measurement of the uptake of [^{14}C]PPAP into the catecholaminergic axon terminals showed that 39.5 mM of PPAP/min/mg protein was taken up by the membrane vesicles. PPAP inhibited the uptake of noradrenaline in the rat cortex homogenate ($IC_{50} = 5 \times 10^{-8}$ M) and was as potent as benztropin in inhibiting the high affinity uptake of dopamine in the rat striatum.

Analysis of the inhibition of the uptake of [^{3}H]noradrenaline by PPAP in purified synaptosomal fractions and in membrane-vesicle preparations from the rat brain revealed that PPAP ($IC_{50} = 1\ \mu$M) was more potent than DMI ($IC_{50} = 16\ \mu$M) in inhibiting the uptake of noradrenaline into the storage vesicles.

In agreement with the biochemical data, measurements on the pulmonary artery strip of the rabbit showed that PPAP inhibited tyramine uptake with an $ID_{50} = 1.22 \times 10^{-6}$ M. It was also highly effective in inhibiting the noradrenaline-releasing effect of PEA *in vivo* in the cat. This was measured via PEA-induced contractions of the cat nictitating membrane, which are of short duration, completely reversible and dose-dependent.

In contrast, methamphetamine, methylphenidate, mazindol, phenmetrazine and amfepramon acted like amphetamine, i.e. they released noradrenaline and elicited long-lasting contractions of the pulmonary artery strip and the nictitating membrane. Thus, the pharmacological data obtained on the pulmonary artery strip of the rabbit and the *in vivo* experiments on the cat nictitating membrane clearly revealed that PPAP is devoid of a catecholamine-releasing property and inhibits the uptake of indirectly acting amines.

Further evidence for the characteristic pharmacological profile of PPAP was furnished by measuring the release of dopamine from the isolated

striatum and tuberculum olfactorium. Amphetamine increased the amount of dopamine released from the axon terminals, whereas PPAP was not only devoid of the releasing property but inhibited the dopamine-releasing effect of amphetamine.

We analyzed the nature of the PPAP-induced stimulation in the CNS with various methods. The motility-increasing effect of PPAP, as well as its stimulatory effect on learning and retention in one-way and two-way avoidance tests, were remarkably different from those of the amphetamines.

Amphetamines are known to be stimulatory in behavioral tests in small doses but they inhibit performance in higher doses. For example, in the shuttle box, 0.5–1 mg/kg of amphetamine significantly improves learning, but 5 mg/kg inhibits performance. The stimulatory effect of amphetamine in the shuttle box is prevented in rats pretreated with α-methylparatyrosine. In contrast, PPAP enhanced performance dose-dependently (2–15 mg/kg) and pretreatment with *a*-methylparatyrosine did not antagonize this effect.

PPAP was highly potent in antagonizing the tetrabenazine-induced depression in the shuttle box and much more effective than amphetamine in the Porsolt test, used as a screening method for predicting the antidepressant effect of drugs.

Considering its pharmacological profile, PPAP may have significant advantages over releasers in long-term clinical use. In contrast to PPAP, releasers induce a continuous overflow of the catecholaminergic transmitters from the neurons, thus dramatically changing the physiological milieu in the brain. The drug-induced release of the catecholamines must necessarily provoke a series of counter-regulations aiming to restore the natural conditions, but all in vain in the persistent presence of the releaser. Amphetamine-dependence might logically be related to the long-term serious disturbances in the delicate physiological equilibriums of the brain amines. Using the Hosoya test, we found in fact for PPAP, free of the catecholamine-releasing property, a reasonably lower dependence capacity in rats (Fig. 5). A more sophisticated analysis of dependence with PPAP, as compared to amphetamine, is now in progress.

The high effectiveness of even low doses of PPAP against the tetrabenazine-induced depression in the shuttle box and its efficiency in the forced swimming test predict a remarkable antidepressant property of this compound.

(−)Deprenyl, the parent compound of PPAP, was tested in Alzheimer's disease and its clinical efficiency is now unequivocally demonstrated (Tariot *et al.*, 1987a, 1987b, 1988; Martini *et al.*, 1987; Piccinin *et al.*, 1990; Mangoni *et al.*, 1991; Goad *et al.*, 1991). An analysis of the pharmacological profile of (−)deprenyl (Knoll, 1992a, 1992b) clearly indicated that this effect might be independent from MAO-B inhibition. (−)Deprenyl is in single doses ineffective in the learning tests (Knoll, 1992c), whereas PPAP is fully effective. All in all, it is reasonable to expect that PPAP will be

effective *per se* and could advantageously be combined with (−)deprenyl in Alzheimer's disease.

Last but not least, the differences in the pharmacological spectrum between PPAP and methylphenidate, another clinically used CNS stimulant with catecholamine-releasing property, deserves attention. Since decades, methylphenidate (Ritalin®) has been a preferred remedy for hyperactive school-age children. For unknown reasons, 2–4 % of the children have serious problems at school because of a short attention span and abnormal distractibility, combined with a hyperactive behavioral status. The well-known syndrome, called since 1987 the "attention-deficit-hyperkinetic disorder" (ADD), is, despite intense research during the last 50 years, still enigmatic (for review see Barkley, 1981; Oades, 1987). Neither is the cause of the therapeutic effect of methylphenidate in hyperkinetic children clearly understood. The mechanism of action being as it may, the pharmacological data induce the belief that PPAP might substitute for methylphenidate. Experimental studies clearly reveal that both PPAP and methylphenidate activate the catecholaminergic system in the brain. There is, however, a basic difference in the mechanisms by which this common final effect is reached. Again, because of the less pronounced changes in free amine concentrations in the brain, PPAP might have advantages over methylphenidate in ADD.

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